A SIMPLE MICROSCOPY TECHNIQUE FOR *ACANTHAMOEBA* IDENTIFICATION

Portia Debono

Introduction

Acanthamoeba, a ubiquitous free living protozoa, has been implicated in a number of diseases, the most serious are *Acanthamoeba* keratitis (Cohen et al., 1987; Moore, 1989) and granulomotous amoebic encephalitis in immunosuppressed patients (Martinez, 1985). Identification techniques of this organism are scanty and nonspecific in Malta. This can easily lead to misidentification of the amoeba, especially by an inexperienced observer (Silvany et al., 1987).

Methology

Survey 1

This consisted of an epidemiological and geographical assessment of *Acanthamoebae* species nasal carriage. Four hundred and six volunteer patients attending the Health Centres of the Department of Health were sampled. Sterile cotton wool swabs, moistened with sterile saline were inserted in the right nostril and rotated firmly against the wall of the nasal vestibule of the patient. The same procedure was repeated in the left nostril with the same swab (Badenoch et al., 1988). The material of the swab was smeared onto two labelled microscope slides, which were then fixed in methanol for one minute and three to five minutes respectively (Ma et al., 1990). The patients were then asked to answer a questionnaire, requesting information about the risk factors associated with nasal carriage or the disease of the amoeba.

Survey 2

A request was sent to 84 hotels in Malta asking for persmission to test a sample from their swimming pools.

An asceptic technique was used (hand's washing and sterile gloves) to collect a surface sample in a sterile 20ml container. The samples were transported at a temperature of approximately 10 degree Celsius (Biddick et al., 1984) to the bacteriology laboratory, where they were centrifuged at 2000 revolutions (Cuschieri, 1991). The residue was transferred on to two glass slides, labelled beforehand. The air dried slides were fixed in methanol as the others in Survey 1. The hotel personnel were given a brief questionnaire concerning the type of disinfecting system; the type of water used and if persons have been in the pool after disinfection.

The same procedure was done on the sea and water polo samples.

Survey 3

Local anaesthetic was instilled in the patient's ulcered eye (Johns et al., 1987; Moore, 1989) and a scraping of the cornea was taken by a sterile blade (Johns et al., 1987; Moore, 1989). The scraping was placed on a slide (Johns et al., 1987; Moore, 1989) without smearing it (Theodore et al., 1985) and the remaining fluid on the blade was smeared on another slide. The slides were fixed in methanol as previously described. In this case the samples were not left to air dry (Ma et al., 1970; Moore, 1989).

Only one sample was attained in this survey.

Staining Procedure

Microscopic controls: The unstained slides were used for training of the staining procedure and as a positive control. Both stained and unstained slides were used to become familiar with the organism. The negative control consisted of methanol cleaned slides, with the same fixative time as that described previously.

The slides, which were immersed in methanol for 3-5 minutes were immersed for 5 minutes in **Cellufluor white** (0.05% **Calcoflour White**; 0.05% **Evans Blue**). The other slides were treated for 10 seconds with **Giemsa-Wright** stain. The slides were screened for the presence of the cysts or trophozoites, under a fluorescent microscope and a light microscope respectively.

Results and Discussion

In all cases the cysts, stained apple-green on the outside. Some had red structures on the inside with a dark nucleus in the centre and with quite visible ostioles on the walls. A star shaped inner cell wall against a smooth round outer cell wall, could be well identified (Ma et al., 1990; Marines et al., 1987).

Survey 1

Amoebae were isolated from 41 subjects giving a point prevalence of 10.1%. This value is high when compared to other prevalence rates such as 1.7% for throat swabs in a New England study (Wang and Feldman,

corneal ulcers of all Contact Lens wearers for *Acanthamoeba* species investigation.

This project urges the population at large and medical staff in particular to be on the alert regarding the importance of strict hygiene, especially with contact lenses. The tendency for the increase of this disease is worrying (Moore et al., 1987), so prevention should be taken seriously. This also applies to other more common causes of keratitis apart from the one mentioned here (Ludwig et al., 1986). However the condition may also result in non contact lens wearers, in which case very little can be done to prevent the disease.

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